SURFACE AREA RESPIRATION DURING THE HATCHING OF ENCYSTED EMBRYOS OF THE BRINE SHRIMP, ARTEMIA SALINA

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When the encysted embryos of *Artemia salina* are placed in water (hydration) the embryos resume development. After an interval of time depending upon conditions of incubation, excystment takes place in two stages. The first stage (emergence) occurs when the hard outer cyst wall splits, and the embryo emerges head-first within a hatching membrane. The second stage (hatching) occurs a few hours later when a nauplius larva swims from the membrane and shell. The transition from the encysted stage to the emerged stage depends upon an uptake of water, mainly due to increased internal concentration of glycerol (Clegg, 1964) and possibly to an increase of free amino acids at the same time (Emerson, 1967). The uptake of water increases the volume of the developing embryo to cause the cyst shell to split. There is consequently an increase of the surface area of the embryo which is shown by scaled micro-photographs of Nakanishi *et al.* (1962).

There are several studies of respiration of *Artemia* during development (Urbani, 1946; Dutrieu, 1960; Muramatsu, 1960; Emerson, 1963; Clegg, 1964). These studies are difficult to compare because of different sources of cysts, possible differences in percentage of viable cysts, and experimental differences in the salinity and temperature of the hatching solution. In spite of differences reported for the rate of oxygen consumption of the embryos, some of these studies reveal a similar pattern. The oxygen consumption rate increases rapidly within the first few hours after hydration, and then remains constant for a time. A second increase occurs at about the time of emergence. Von Bertalanffy and Krywienczyk (1953) have shown that oxygen consumption of the nauplius and later stages of *Artemia* is proportional to surface area. An increase of surface area during emergence could account for the increase of respiration which occurs at the same time. The present study demonstrates that oxygen consumption patterns of developing *Artemia* embryos can be interpreted on the basis of surface rule respiration.

Materials and Methods

1. Source of encysted Artemia embryos

The encysted embryos used in this study were obtained in 1965 from Ward's of California (Monterey). The cysts were from Great Salt Lake, Utah.

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2. Respiration measurements

Oxygen consumption of the *Artemia* embryos was measured with a Warburg constant volume respirometer (Umbreit *et al.*, 1959). Dry cyst samples weighing 10.0 mg. were placed in flasks (18 ml. volume) with center well and sidearm. The flasks contained 2.5 ml. 0.5 *M* NaCl, and 0.2 ml. 20% KOH in the center well. Readings were made at 1–2-hour intervals at 25° C. Calculations are expressed as μl. O_2/hr./mg. dry cyst weight. Most of the experiments were carried out with no agitation of the flasks and no antibiotics in the water. Since somewhat different readings were obtained than in a similar set of experiments (Emerson, 1963), other series of measurements were made with agitation at a rate of 60 complete oscillations/min., and with antibiotics in the water (penicillin, 1000 units/ml. and streptomycin, 100 μg./ml., Clegg, 1964). Microbial activity was evaluated at the end of runs in which there were no antibiotics in the water by filtering off the brine shrimp (Whatman #1 paper) and measuring oxygen consumption of the water over a period of several hours.

3. Measurement of surface area of cysts and of emerged embryos

All measurements were made with a dissecting microscope fitted with a calibrated eyepiece micrometer. The encysted embryos are spherical in shape. Surface area was calculated directly from measurements of diameter, using the formula for the surface area of a sphere (area = 12.57 r^2, where r = radius). The emerged embroys have a symmetrical shape resembling a pear. Measurements of emerged embryos were drawn on graph paper. Each drawing was divided into sections. Surface areas of the middle sections were calculated using the formula for the curved surface of a right cylinder (area = $2\pi rh$, where h = altitude). The surface areas of the two end sections were calculated as the curved surface of a right cone (area = $\pi r\sqrt{r^2 + h^2}$). Areas of individual sections were totaled to give the surface area of the emerged embryo.

4. Percentage of emergence

The period of time when 50% of the embryos were fully emerged ($T_{50\%}E$) was estimated by periodic counts of the percentage of emerged embryos during development.

Results

Oxygen consumption rates during development are summarized in Table I. The presence of antibiotics in the incubation media, or agitation of the Warburg vessels does not significantly affect oxygen measurements (Table II). The surface areas of encysted and emerged embryos are compared in Table III.

Discussion

The following terms are used to describe the oxygen uptake pattern of *Artemia* embryos during development (Table I). The hydration period is the first rapid uptake of oxygen; the differentiation period is a plateau during which the rate of oxygen consumption remains about the same; the emergence period occurs during the second rise of oxygen consumption rate when most of the embryos are emerging;

TABLE 1

Oxygen consumption of developing Artemia embryos in 0.5 M NaCl at 25° C. The values given are μl. O_2/hr/.mg. dry cyst weight. The numbers preceded by ± signs give confidence limits at the 95% level

Hours of development	Oxygen consumption (15 determinations)	* Period of development
0	———	
1–2	0.46 ± 0.08	Hydration
2–4	0.93 ± 0.02	
4–6	1.06 ± 0.05	
6–8	1.05 ± 0.11	
8–10	1.17 ± 0.17	
10–12	1.10 ± 0.17	Differentiation
12–14	1.20 ± 0.03	
14–16	1.01 ± 0.15	
16–18	1.21 ± 0.23	
18–20	1.44 ± 0.23	
20–22	1.66 ± 0.36	** Emergence
22–24	1.73 ± 0.36	
28–30	1.94 ± 0.42	
30–32	2.00 ± 0.49	Hatching
36–38	2.00 ± 0.26	

* See text for explanation.

** The first emerged embryos were seen at 16 hours; $T_{50\%}E$ was at 24 hours.

and the hatching period is when oxygen consumption levels off again after $T_{50\%}E$ Average rates of oxygen consumption of the differentiation and of the hatching period of this study are compared with other studies (Table IV).

Muramatsu's measurements went only to 12 hours of development so that the hatching period probably was not reached. Urbani's measurements probably represent oxygen consumption well past $T_{50\%}E$, since the figure listed under hatching period (Table IV) is a value for 50 hours of developing. These two studies will not be considered in the following discussion.

TABLE II

Comparison of conditions for oxygen consumption measurements of developing Artemia embryos through 20 hr. development in 0.5 M NaCl at 25° C. The numbers in parentheses indicate the number of determinations. The numbers preceded by ± signs give confidence limits at the 95% level

Condition	Total μl. O_2/mg. dry cyst (20 hr.)
*No antibiotics; not agitated (15)	20.8 ± 1.3
Penicillin and streptomycin; not agitated (6)	19.9 ± 3.0
Penicillin and streptomycin; agitated at 60 complete oscillations per minute (6)	19.7 ± 2.2

* Measurement of filtered water at the end of the runs showed very little oxygen consumption due to microbial activity.

TABLE III

Surface area of Artemia embryos during development

Stage	* Surface area (μ^2)
Encysted embryo (differentiation period)	119,415
Fully emerged embryo (hatching period)	205,178
% increase of surface area	172%

* Averages of 10 measurements. Statistical variation is not shown because individual measurements were almost identical.

The increase in oxygen consumption (Table IV) is very similar to the increase of surface area (Table III) during emergence. This observation suggests that the increase of oxygen consumption rate is proportional to an increase of surface area. The pattern of oxygen consumption (Table I) can be interpreted as follows: Oxygen consumption rises during the hydration period (1–3 hours in duration; Iwasaki, 1964) due to reactivation of metabolism of the dormant embryo. The initial rate rises to a constant value which is limited by the surface area of the cyst throughout the differentiation period. During this period, there is no cell division (Nakanishi *et al.*, 1962; Emerson, 1963), no increase of DNA (Bellini, 1960; Emerson, 1963); and no incorporation of tritiated thymidine (Emerson, 1963). Tritiated thymidine is incorporated only after hatching (Emerson, 1964) as cells start to divide (Nakanishi *et al.*, 1962, 1963). The respiratory quotient remains close to 1 during this period, indicating metabolism of carbohydrate (Dutrieu, 1960; Muramatsu, 1960; Emerson, 1963; Clegg, 1964) which is probably trehalose (Dutrieu, 1960). The rate of oxygen consumption increases during emergence, and rises rapidly to a new peak limited by the surface area of the emerged embryo and early nauplius. The respiratory substrate during and after emergence is probably lipid as indicated by lowered respiratory quotients (Dutrieu, 1960; Emerson, 1963), and increase in lipase activity (Bellini and Lavizzari, 1958) and a decrease in total lipids (Dutrieu, 1960; Urbani, 1959).

The present study shows that surface rule respiration can explain the pattern of oxygen consumption during development of encysted *Artemia* embryos. Similar patterns of respiration exist during the embryonic development of a variety of animals (Boell, 1955). It would be interesting to see if surface rule respiration

TABLE IV

Average rates of oxygen consumption of the differentiation and of the hatching period of Artemia

Reference	μl. O_2 consumed per hour		% increase
	Diff. period	Hatch. period	
Table I	1.11 μl./mg.	1.98 μl./mg.	178
Emerson, 1963	1.66 μl./mg.	2.95 μ./mg.	178
Dutrieu, 1960	1.30 μl./mg.	2.23 μl./mg.	172
Muramatsu, 1960	1.03 μl./mg.	1.45 μl./mg.	141
Urbani, 1946	0.00009 μl./cyst	0.00019 μl./cyst	211

applies for these animals, especially for sea urchins which have strikingly similar patterns (Lindahl, 1939; Wright, 1963).

Summary

Oxygen consumption of *Artemia salina* was measured during development in 0.5 *M* NaCl at 25° C. A pattern is seen in which the rate of oxygen consumption increases rapidly within the first few hours after hydration, remains constant for a time, and then increases rapidly again while most of the embryos are emerging. This pattern is dependent upon surface area of the developing embryo. During emergence, the surface area of the embryo increases 172% over the surface area of the encysted embryo. During the same development period, oxygen uptake increases by almost the same factor.

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